Miller (W. D.) Die Mikroorganismen der Mundhöhle. 8vo. *Leipzig* 1889. The Author.

Parlatore (F.) Flora Italiana. Vol. IX. 8vo. *Firenze* 1890. Prof. T. Caruel.

Richardson (B. W.), F.R.S. National Health. Abridged from "The Health of Nations," a Review of the Works of Sir Edwin Chadwick, K.C.B. 8vo. *London* 1890. With Two Pamphlets. 8vo. Sir Edwin Chadwick.

Spencer (J. W.) The Iroquois Beach, a Chapter in the Geological History of Lake Ontario. 4to. [*Montreal*] 1889. The Author.

Waterhouse (J.) Annual Address and Review. 8vo. *Calcutta* 1890. The Author.

"On the Germination of the Seed of the Castor-oil Plant (*Ricinus communis*)." By J. R. Green, M.A., B.Sc., F.L.S., Professor of Botany to the Pharmaceutical Society of Great Britain. Communicated by Professor M. Foster, Sec. R.S.

Received January 29,—Read January 30, 1890.

The germination of those seeds in which the non-nitrogenous reserve material is found to be an oil and not a carbohydrate was first studied by Sachs, who, in a series of papers published in 1859,* put forward a hypothesis to explain the manner in which the oil becomes available for the nutrition of the young plant. He records as his starting point the observation that at the onset of germination the oil gradually disappears, just as starch disappears from the reservoirs when germination is started in a seed containing it, and he shows that the development of the young plant proceeds concurrently with this disappearance. From this it follows that the oil, like the starch, is a reserve material to be made use of by the embryo in its early growth. So far as he deals with the changes taking place in the oil, he puts forward the view that starch is directly formed from it, and that this conversion is the first step that may be traced. Subsequently, sugar arises from the starch, and thus, in all seeds alike, the non-nitrogenous reserves travel as sugar from their storehouses to the seat of growth.

Though putting forward the view of the conversion of fat directly into starch, he appears to be scarcely satisfied with it, speaking of it as being very surprising, and, indeed, admitting that in many seeds the greater portion of the disappearing oil gives rise immediately to sugar, and not through the intervention of starch.

Sachs's view that fat or oil is transformed into starch was soon

* 'Bot. Zeitg.,' 1859, col. 178 *et seq.*

after endorsed by Peters,* who published comparative analyses of the oily seed and seedling of the pumpkin. By these tables, Peters shows that during the early period of germination the oil diminished from about 50 per cent. of the weight of the seed till it only amounted to 17 per cent., and that starch appeared in the same time in quantity equal to 4 per cent. of the weight of the seed. During a second period the oil went down from 17 to 11 per cent., while the starch increased from 4 to 7·6 per cent. During a third period, while the oil sank to 4 per cent., the starch also diminished, becoming rather less than 3 per cent. Peters, in considering these analyses, appears to hold the view put forward by Sachs, that the starch is produced by the transformation of the oil, though the quantities given do not at all necessarily bear out the hypothesis.

Fleury,† in 1865, working on the castor-oil, rape, and almond plants, denied the necessity for the occurrence of starch, pointing out that as the oil disappears sugar is to be found. He further noticed that during the germination there was a formation of a non-volatile acid in small quantity.

V. Hellriegel,‡ who investigated rapeseed, agrees with Fleury in denying the occurrence of starch as an intermediate product of the conversion of fat. He points out that germination is attended by processes of oxidation, there being an evolution of CO_2 during the whole period.

In 1871, a new fact was noted by Müntz,§ that during germination a quantity of fatty acid appears in the seed, pointing to a splitting up of the oil into such fatty acid and glycerine. Müntz suggests that the embryo may play a very important part in such a transformation, acting after the manner of a ferment. He failed to find any glycerine in any of his experiments, but says that the fatty acid increases in quantity as the germination proceeds. The fate of the glycerine, if liberated, he did not trace, nor does he suggest what becomes of it.

Schützenberger,‖ in 1876, drew attention to the fact that if oily seeds be steeped in water, an emulsion is obtained in which very soon may be noted the appearance of glycerine and fatty acid, and puts forward the view that during germination an emulsive and saponifying ferment, placed with fat in the presence of water, causes it to undergo true digestion and renders it assimilable. He gives, however, no evidence of the existence of such a ferment.

* Peters, 'Landwirthsch. Versuchsstat.,' vol. 3, 1861.

† 'Annales de Chimie,' ser. 4, vol. 4, p. 38.

‡ Detmer, 'Vergleichende Physiologie des Keimungsprocesses der Samen.' Jena, 1880, p. 334.

§ Müntz, 'Annales de Chimie,' ser. 4, vol. 22, 1871.

‖ 'On Fermentation' (Internat. Scientif. Series, vol. xx).

Detmer,* in 1880, in criticising Müntz's results, quoted above, endeavours to reconcile the decomposition suggested by the latter with the older statements of Sachs as to the origination of starch at the expense of the oil. He suggests that the glycerine may be transformed into certain unknown bodies, and that the fatty acid may be the immediate antecedent of the starch, giving as a possible explanation of the transformation the equation

$$C_{18}H_{34}O_2+27O = 2C_6H_{10}O_5+6CO_2+7H_2O.$$

He had himself, in 1875, published some analyses of hempseed before and after germination, showing that with a disappearance of about 16 parts of oil there was a formation of nearly 9 parts of starch.

In his later work, in 1882, Sachs† somewhat modifies the views originally propounded by him in 1859, though still adhering in the main to his hypothesis that at any rate the greater part of the oil is transformed directly to starch and sugar. He remarks upon the occurrence of oil drops in considerable quantity in the parenchyma of the roots and shoot-axis of the seedlings of *Ricinus* and other plants, and admitting the possibility of the accuracy of Schützenberger's suggestion of ferment action, he thinks that these fat globules may be due to the recombination of the fatty acid and glycerine, which may travel separately from cell to cell, recombining under certain conditions. Such a process presents a certain similarity with the movements of transitory starch.

On the hypothesis of ferment action he suggests that the ferment is formed in the cotyledons and excreted from them into the endosperm.

During all this period, our ideas of the actual transformation of the fatty matter have rested upon hypothesis rather than experiment. Sachs's first work only noted the coincident disappearance of oil and appearance of starch. He does not say that the two processes go on together in the same cells nor even in the same regions of the seed, the starch in *Ricinus* appearing most copiously in the embryo, the oil vanishing from the endosperm. Nor has the ferment, suggested by Schützenberger, been identified, the chief argument for its existence having been derived from Hoppe-Seyler's description of a very similar change in the fats during pancreatic digestion by animals.‡ Nor have we, beyond Detmer's hypothesis already quoted, any attempt to trace the fate of the glycerine and fatty acids formed. The whole question of the mode of the absorption of the fatty reserves of the endosperm by the embryo and their way of passing

* *Op. cit.*

† Sachs, 'Vorlesungen über Pflanzen-Physiologie,' 1882. English Edition. Ward, 1887, p. 347.

‡ Detmer, *op. cit.*, p. 338; Vines, 'Physiology of Plants,' p. 190.

from cell to cell has also remained unexplained, except by Sachs's first hypothesis of their ultimate conversion through the form of starch into sugar, and by a suggestion he makes that some of the oil at least passes as such through the cell walls of the endosperm into the cotyledon.

In taking up the subject where it had thus been left by previous investigators, and endeavouring to throw further light on the several steps that had been established by them, I commenced to work upon the seed of the castor-oil plant (*Ricinus communis*), and I set before me the following problems :—

1st. By what agencies are the reserve materials made available for the nutrition of the embryo ?

2nd. In what condition and by what process do they undergo absorption ?

3rd. What parts are played in the process of germination by the endosperm and by the embryo respectively ?

I selected *Ricinus* as the material to work upon, because the contents of the seed have been accurately ascertained, and found to contain a large quantity of oil ; and because the process of germination is not prolonged. A few of the points that arose during the work I also investigated on the seed of the cocoanut palm (*Cocos nucifera*).

The seeds of the castor-oil plant consist of a central embryo, embedded in a mass of endosperm, the whole incased by a hard testa. The micropyle is protected by a well-developed caruncle. A section of the endosperm shows the cells to be nearly filled with oil, which exudes on pressing the bruised seed. This oil can be removed by solvents, when the cells are found to contain also stores of proteid in the form of aleurone grains embedded in protoplasm, the grains containing each a crystalloid of proteid and a small aggregation of mineral matter. Starch is not present, nor is more than a trace of sugar to be found, and this small amount is not constant. There is no great amount of cellulose, the cell walls being very thin and the cells of fair size. No glucoside can be detected in the cells.

The amount of oil to be found is stated differently by different observers. According to Harz,* the quantity varies from 40 to 68 per cent., the highest figure being quoted by Cloez. Blumenbach puts it at 50 per cent. I found a fair sample of the seeds to yield 58 per cent. of their dry weight. The oil is somewhat complex, yielding, on saponification, according to Lecanu and Buffy,† three fatty acids, ricinstearic, ricinic, and ricinoleic, of which the latter is the most abundant. It has the composition represented by the formula $C_{18}H_{34}O_3$.

* 'Landwirthsch. Samenkunde,' 1885, p. 836.

† Harz, 'Landwirthsch. Samenkunde,' 1885, p. 836.

The proteids were investigated in detail by Vines,* who speaks of them as consisting of members of the peptone and globulin classes, the former being soluble in water, the latter in solutions of NaCl.

I. *Agencies by which these reserve materials are made available for the use of the Embryo.*

The first two problems divided themselves at once into two sections, the first of which involved the changes in the oil, the second those in the proteids. They were investigated separately.

(i.) *The Oil.*

Both Müntz and Schützenberger had suggested the probability of the changes in the oil being due to the action of a ferment. My own work on the proteolytic changes in the germination of the lupin† proved a similar cause in connexion with the proteids of that plant. In endorsing Schützenberger's suggestion, Sachs, in 1882,‡ put it forward as probable, from his investigations on the seed of the date (*Phœnix dactylifera*), that the embryo of the young plant, on the onset of germination, formed and excreted ferments to change the reserve materials into a form suitable for absorption.

Some seeds of *Ricinus* were germinated for five days, until the young plants had developed a good root system, and about half the endosperm had disappeared. The seeds were then taken up, the endosperms separated from the cotyledons, and the cotyledons from the hypocotyledonary portion of the embryo. Extracts were made of the endosperms and of the cotyledons, the fluids used being 5 per cent. salt solution and glycerine, in both of which ferments are known to be soluble. Putrefaction was obviated when the salt solution was used by the addition of 0·2 per cent. of potassic cyanide, which was found efficacious in preventing the appearance of bacteria. In the course of the investigation several such extracts were made, the salt solution ones being, on the whole, the most satisfactory.

Experiments were then made to ascertain whether, in the extracts, anything was present which was capable of starting chemical changes in the oil, and, if so, whether that body was of the nature of a ferment. The change first to be expected was the splitting up of the oil into fatty acid or acids and glycerine. 5 c.c. of the extract of the endosperms was mixed with 10 c.c. of an emulsion of castor oil, and set aside in a test-tube in a bath or incubator, at a temperature of 37° C. A control was kept by preparing a similar tube after boiling

* 'Journal of Physiology,' vol. 3, pp. 93—114.

† 'Phil. Trans.,' B, 1887, pp. 39–59.

‡ *Op. cit.*

the 5 c.c. extract. No change was apparent for some hours, but gradually the unboiled tube became acid, while the control remained unchanged. Different extracts varied somewhat in the amount of acidity thus caused, but the difference between the boiled and unboiled tubes was soon evident. A fairly typical experiment is subjoined:—

Tube F was prepared by mixing the extract and the emulsion of castor oil in the proportions given above, and was put into an incubator at 12.30 o'clock on August 22, 1888. A boiled control was put with it, labelled F_1. Both were carefully made neutral. At 4 P.M. 10 drops litmus solution were added to each. F was acid, F_1 neutral. The degree of acidity was in this short time only slight, and addition of 0·1 c.c. of Na_2CO_3 solution of 0·3 per cent. strength again neutralised it. Replacing the tubes in the incubator and leaving them till next morning, F had again become acid. A further addition of the same alkali was made to the contents of this tube, and neutrality was obtained when 1·75 c.c. had been added. The action had been, during the longer exposure, more than proportionately vigorous. No change took place in the control tube.

A similar set of experiments with the extract of the cotyledons failed to produce any evidence of ferment action, the reaction of the liquids always remaining unchanged.

So far as the oil is concerned, therefore, the experiments confirm the hypothesis of Schützenberger, that *there is a ferment in the seeds which can develop fatty acid from the oil.*

Another experiment was then carried out, extending over a longer time, dialysing tubes being used instead of glass vessels. The extracts were prepared by salt solution (5 per cent. NaCl + 0·2 per cent. KCN), and were dialysed before use till nearly free from NaCl, this being found to hinder the action somewhat. The proportion of KCN was kept constant during the experiment.

One fluid ounce of castor oil was made into a thick emulsion with gum and 10 c.c. of the dialysed extract stirred into it. This was put into a dialysing tube and suspended in 200 c.c. of a solution containing 0·6 per cent. NaCl and 0·2 per cent. KCN (No. 1). A control was prepared in the same proportion, boiling the 10 c.c. extract (No. 2). The two were kept in the incubator for a week. During this time the emulsion in No. 1 became gradually purple, and then reddish; that in No. 2 remained blue. The reactions of the dialysates did not change.

On concentrating the two dialysates at the end of the experiment, glycerine was detected in that of No. 1, while No. 2 contained none.

The ferment in the extract had liberated fatty acid and glycerine, and both had become traceable. It was noteworthy, too, that the body causing the acidity had not passed through the dialyser.

The ferment found, therefore, like the ferment in the pancreatic juice of animals, is capable of decomposing fats into fatty acids and glycerine.

The activity of this ferment, like that of so many others, was found to be largely influenced by the reaction of the medium in which it was caused to work. A set of experiments on this point is subjoined:—

Five tubes, A—E, were taken, and in each 5 c.c. of a ferment extract, carefully neutralised, were set to work on 5 c.c. emulsion of castor oil. They were made of different reactions, as under:—

A	contained	0·066	per cent.	HCl.
B	„	0·133	„	„
C	„	0·066	„	Na_2CO_3.
D	„	0·66	„	„

E was left neutral, as much water being added to it as was added to each of the others with their respective acid or alkali. A_1, B_1, C_1, D_1, E_1 were controls prepared like the others, but with boiled extract.

After $3\frac{1}{2}$ hours they were examined by the addition to each of 10 drops solution of litmus, when A and E became pinker than their controls, B about equal to B_1. C was less alkaline than C_1, but the D set were relatively unchanged. Titration of the tubes with weak alkali as before showed that *the ferment works best in neutral solution, is hindered by as little as* 0·066 *per cent. of HCl, and stopped by* 0·133 *per cent. of HCl. It is hindered by alkalis also, but works quite well in them if the solution is weak,* 0·066 *per cent. of Na_2CO_3 only retarding the action slightly;* 0·66 *per cent., however, stopped it entirely.*

Neither the 0·133 per cent. HCl nor the 0·66 per cent. Na_2CO_3 destroyed the ferment, for, on neutralising the four tubes, B, B_1, D, D_1, and allowing them to stand in the incubator for several hours, action began again in both the unboiled ones. The energy of the two was, however, very different, for the acid liberated by the ferment in D was five times the amount set free in B in the same time. The activities of B, D, and E, tested during eighteen hours, are represented by the following figures:—

B.	D.	E.
Exposed to 0·133 per cent. HCl for $3\frac{1}{2}$ hours, and then neutralised.	Exposed to 0·66 per cent. Na_2CO_3 for $3\frac{1}{2}$ hours, and then neutralised.	Kept neutral all the time.
10.	50.	85.

The ferment was by similar experiments found to be absent from the resting seeds. Analogy with other ferments of both animal and vegetable origin pointed to its probable existence here in the form of a zymogen. To test this point, several experiments were made. Some resting seeds were ground and treated with ether to extract

the oil. Half the powder was then extracted by the usual solution of NaCl and KCN, while the rest was extracted in the incubator by weak acetic acid, only so strong as to be just perceptible by the tongue. These two extracts were filtered, and labelled respectively A and B, B being then carefully neutralised. Part of A was then acidified with weak acetic acid, and warmed to 35° C. for three hours, and then neutralised. This was labelled C.

On testing them all with castor oil emulsion in the usual way, side by side with boiled controls, A developed no acid, B and C both showed its liberation, giving evidence of the development of ferment by the weak acetic acid. This behaviour is precisely similar to the influence of the same reagent on extracts of the resting pancreas. On allowing extract A to stand in the laboratory for nine days and then again testing it, there was evidence of the presence of ferment, liberated by keeping it. It was not, however, so active as extract C, where the transformation had been brought about by the acetic acid.

(ii.) *The Proteids.*

The oil is only a part of the reserve material present in the seeds, and as so much proteid matter also is present in their cells, some experiments were made to see if a further ferment is present having a proteolytic function.

The proteid in the seed had been stated by Vines* to be a mixture of peptone and globulin, the crystalloid consisting of the latter.

Some ground resting seeds were extracted with water and subsequently with salt solution. Both extracts were found to give a coagulum on boiling, which was most copious in the second case, and separated out completely if a little HNO_3 was added. This was Vines's globulin. After filtering off this precipitate while hot, another proteid was found to be left in solution, which was precipitated as the liquid cooled, and redissolved when it was warmed again. There was much less of this than of the first one. No dialysable proteid was present. The first proteid found by Vines was therefore not a peptone, as he supposed, but an albumose.†

The method adopted in searching for a proteolytic ferment in the germinating seeds was exactly the same as the one I had used in the case of the lupin,‡ and the results showed that again here, as there, a tryptic ferment is developed during germination, which can split up fibrin with formation of peptone and crystalline bodies, including tyrosin.

* *Loc. cit.*

† *Cf.* Martin "On the Proteids in Papau-juice." 'Journal of Physiology,' vol. 6, p. 354.

‡ 'Phil. Trans.,' B, 1887, pp. 39—59.

Thus *the germinating seeds are seen to contain at least two ferments, both working towards the utilisation of the reserve materials in the nutrition of the embryo.*

The glyceride ferment is capable only of producing fatty acid and glycerine from the oil. The changes cannot, however, end with this decomposition. Fleury had noticed in 1865* that there was free acid formed during germination. Müntz had pointed out that the reaction of the embryo was strongly acid, and that at no stage in the germination could he detect glycerine in any of the tissues.† Again, sugar is present in the germinating endosperms in abundance.

Before making experiments on the possible decompositions of the bodies resulting from the action of the ferments, it seemed advisable to examine more carefully the contents of the endosperm. Some seeds were germinated till the endosperms were on the point of separating from the cotyledons; they were then removed, and the endosperms ground up in a mortar with distilled water. This solution was strongly acid. It filtered nearly clear, but was a little opalescent, which appearance was found under the microscope to be due to very minute drops of oil or fatty acid. The solution was then dialysed with distilled water for two days, when the dialysate was found to be acid in reaction. The power of dialysis of the free fatty acids was presumably small, they being extremely greasy liquids, very much like the oil itself. Careful experiments showed that they had no power of dialysis, or at most an extremely feeble one, whether tested as they were or made up into an emulsion. After two days' exposure in a dialyser, the outside liquid had the merest trace of acidity. The experiment noted above,‡ when the digestion was carried out in a dialyser, also negatives the theory of their being able to pass a membrane, for, though glycerine passed out during the experiment, the action of the dialysate remained neutral. As, then, ricinoleic acid will not dialyse, there was evidently another acid in the germinating endosperm. The dialysate of its extract was next evaporated to dryness on a water-bath, and the residue extracted with ether. A certain amount of insoluble matter was left, and the extract therefore filtered. On slowly evaporating the ether, it deposited a crystalline residue, which was freely soluble in water with a resulting acid reaction.

An extract of the cotyledons gave exactly similar results. This new acid was really the cause of the acid reaction of the whole of the tissues of the young plant, already shown to exist by several of the writers already quoted.

The extracts themselves, of both endosperms and cotyledons,

* *Op. cit.*

† *Op. cit.*

‡ P. 375.

deposited on evaporation a residue which gave up to ether a little oily matter, as well as this crystallisable acid, differing thus from the dialysates. In both extracts evidently there existed a mixture of the crystallisable acid with oil and fatty acid.

The fatty acids are also present in the cells. A further quantity of the germinating endosperms was extracted by ether for two days, and the liquid decanted and filtered. A greasy residue was left, the greater part of which was insoluble in water. Unlike the oil, it was largely soluble in 0·2 per cent. NaHO. A solution in this reagent, when neutralised by HCl, gave a white curdy precipitate. This was soluble again in more alkali, and was again thrown down on acidifying. This behaviour was exactly reproduced by some pure ricinoleic acid supplied by Schuchardt.

The extract of the cotyledons contained a little, but not much, of this fatty acid. It seemed likely that a good deal of the contents of the disintegrating endosperm cells might be adhering to the cotyledons, and another experiment was therefore made, using cotyledons that had been carefully washed in 0·2 per cent. NaHO to remove any such *débris*. This weak soda solution, after the washing, gave an opalescence with HCl, and the extract made of the washed cotyledons was found to contain a very faint trace of the fatty acid.

The germinating endosperms, tested with iodine under the microscope, were found to contain no starch. There was starch only in certain regions of the young embryo. On testing a piece of the germinating endosperm with Fehling's fluid a copious reduction was observed. The extracts made and used as described above also gave evidence of the presence of sugar. In the extract of the ungerminated seeds very much less was present. 22 grams of these were found on careful titration to contain 0·025 gram, or 0·11 per cent. of sugar, calculated as dextrose. The quantitative examination of the germinating seeds showed a great increase, which appeared to proceed side by side with the disappearance of the oil. 18 grams of dry weight of endosperms were taken and thoroughly extracted by ether. The seeds from which the endosperms were picked had a root now about 3 inches long, with secondary roots attached. The loss of weight after the ether extraction was 4·7 grams, or about 26 per cent. The ether extracted not only the oil that remained unchanged, but certain of the products of its decomposition, viz., the fatty acid and the crystallisable acid then present in the endosperm. The resting seeds contained 58 per cent. of oil, so that 32 per cent., calculated on the weight of the endosperm, or 55 per cent. of the total oil, had disappeared, while some of that which was still traceable had been at least partially decomposed. The endosperm was next thoroughly exhausted with boiling absolute alcohol, which extracted all the sugar present. This was evaporated to a syrup, dissolved in water,

and titrated, when it was found to contain 0·174 gram of sugar, reckoned as dextrose, or 0·97 per cent. of the original dry weight. Without taking into account the amount that had been absorbed, which must have been very considerable, as the young plant had attained a great degree of development, there was still present almost ten times as much as in the resting seed.

Examination of the proteid constituents in the germinating endosperm, showed that in addition to the undecomposed proteids found in the resting seeds peptone was present. The quantity obtainable from different extracts was not uniform, some being very rich and others containing but little. The cotyledons were removed from some of the advanced seeds and extracted with water in dialysers. After two days the dialysates were concentrated to small bulk, and acetate of zinc added. This caused a precipitate, which was filtered off, washed, and suspended in water. The zinc was removed by H_2S, and the watery solution concentrated again, when it deposited crystals of asparagin.

The endosperm, examined in the same way, was found to contain no perceptible amount of this substance.

On examining some of the endosperms after a prolonged period of germination, carried indeed so far that there was only a thin, almost slimy casing over the cotyledons, the cells were found to be empty of solid contents, except a thin layer of protoplasm, and the cell walls were disintegrating and disappearing. No substances could be extracted now, except a small amount of sugar and some of the crystallisable acid described above. Absorption was still proceeding, though the young plant had attained a considerable development.

The germinating endosperm was thus found to contain oil, free fatty acid, a crystallisable acid, a greatly increased quantity of sugar, peptone, and unaltered proteids. No glycerine was present. The embryo contained also a certain amount of asparagin. These bodies are not all due to the action of the ferments. The latter cannot decompose the fatty acid, nor produce the dialysable one. Nor can the sugar be traced to its activity. The first problem then is partially solved by the identification of the ferments, but the work of the glyceride one, at least, needs supplementing by further activity connected with vital processes taking place in the endosperm cells under conditions to be discussed later,* and under the influence of the protoplasm of these cells.

II. *Mode of Absorption of the Reserve Materials.*

The form and manner in which these different reserve materials are absorbed has, as before mentioned, been the subject of hypothesis.

* *Cf.* p. 384 *et seq.*

Sachs* held that, as the cells of the cotyledons of *Ricinus* contain oil after germination has commenced, this oil must have the power of passing through cell walls, and even through the epidermis of the cotyledons.

Detmer,† on the other hand, suggests that, like starch, it becomes a material capable of dialysis, and travels by such means, the oil which appears in the cotyledons and other parts being due to a re-formation at the spot at which it is found, as is the case with transitory starch.

The structure of the cotyledon shows that the actual passage of oil into it would be a matter of very great difficulty. Its outer epidermis is separated from the cells of the endosperm by a very thick layer of cell walls, the remains of cells whose contents have been absorbed; and microscopic examination of this layer while absorption is proceeding fails to detect any fat in its thickness. All analogy points too to dialysis as the mode of absorption, and the forms in which the reserve materials are to be found in the endosperm during germination indicate such a process as the probable one.

Of the various bodies found, sugar and the crystallisable acid are easily capable of dialysis, and are obtainable from the cotyledons. Peptone also can pass through a membrane. Asparagin can be detected on the cotyledonary side, but not in the endosperm. Peptone, however, is not to be detected in the cotyledons.

Besides these, there are to be found in the young plant a certain amount of oil, some considerable quantity of starch, and a trace of fatty acid.

The form of absorption of the nitrogenous matter seems to be that of asparagin, for peptone, though formed, does not seem to leave the endosperm. This is in accordance with the condition in the lupin,‡ where the nitrogenous matter travels in the same way, peptone being only a stage in its formation. The fact that asparagin is not traceable in the endosperm is not a valid objection to this view, for it is quite possible that it is absorbed as fast as formed, or that so little is left behind that it escapes observation.

Detmer's hypothesis seems to account satisfactorily for the appearance of the oil in the young plant, and, if valid, it explains also the trace of fatty acid there. The latter could only be explained otherwise by its having, either in the free state, or in the form of one of its salts, the power of dialysis. Experiments already recorded negative the idea of its dialysing in the free state. Some careful investigations were made as to the behaviour of its alkaline salts. Some ricinoleic acid was made into soaps with different strengths of

* *Op. cit.*, p. 347.

† *Op. cit.*, p. 370.

‡ Green, *op. cit.*

soda solution, and these were dialysed for two days in freshly tested dialysers. I expected to find that, instead of dialysing intact, there would be a decomposition, and that the fatty acid would be left behind, while the alkali escaped. Contrary to this expectation, the dialysates in all cases gave a marked opalescence, or a curdy precipitate, with HCl, the ricinoleic acid being liberated thereby from soap which had passed the dialyser. Subsequent careful examination of the dialysing tube proved it to be intact. The experiment, though not without interest, does not throw any light on the mode of absorption of the acid, for, on neutralising or making slightly acid, the soap is decomposed, and the fatty acid liberated. As the reaction of both endosperm and cotyledon is acid, it is clear that the fatty acid does not pass from the one to the other in the form of a soap.

The occurrence of the starch must be similarly explained. It results, as in other plants, from the transformation of the sugar which has been absorbed. In sections of the hypocotyledonary portion of the axis I found some roundish bodies, which when heated with iodine stained brown. With a $\frac{1}{10}$ objective these brownish bodies were seen to contain small crescent shaped bodies which were dark blue. Some contained two, others three or four, of these. There is little doubt that these were amyloplasts, containing starch grains of very small size, but in course of formation. I did not succeed in identifying these in the cotyledons, though starch appeared there in small grains. Gris* has figured bodies exactly corresponding to these, and he says he finds them in the cells of the epidermis of the cotyledon.

An examination of the relative quantities of these different conditions of the fatty reserve materials present at different stages of the germination confirmed the view given above. The disappearance of oil and coincident increase of sugar have already been commented on. The relations between the oil and the fatty and crystallisable acids were separately determined. Some seeds were germinated in an incubator, and samples were examined at intervals of twenty-four hours from their being sown. Care was taken to have all the seeds of about the same size, and as much alike as possible. Two sets of experiments were made, in one the quantities of the different constituents of the whole seed and resulting plant being examined—in the other those in the endosperms alone.

Each sample was crushed and extracted by ether, the extract being evaporated to dryness, and the amount of residue roughly estimated. This residue was then stirred well with water, which was syphoned off, and added for twenty-four hours to the remains of the crushed endosperms now freed from the ether. The extract so obtained was examined for crystallisable acid and for sugar. The

* 'Ann. des Sci. Nat.,' Ser. 5, *Bot.*, vol. 2, 1864.

residue left by the ether, after being washed with water as described to remove any soluble acid, was treated with weak alkali (0·2 per cent. NaHO) to remove fatty acid, and this alkali afterwards acidified by HCl to set free the fatty acid from the soap formed. Coincidently with each experiment, endosperms of the same age were examined for glycerine.

The following tables give the results of the experiments:—

Table I.—Whole Plant examined.

Time germinating.	Degree of development of embryo.	Residue deposited by the ether.	Oil found in same.
hours 24	Seeds just cracking testa	Copious, greasy......	Bulk of residue.
48	Primary root 1 cm. long	"	75—80 per cent.
72	Lateral roots emerging	Slightly less in bulk, greasy	50 per cent., about.
96	Root system spreading	Still less. Water mixed with the fat	20—30 per cent.
120	Large root system...	Less still. Getting watery	About half last quantity.
168	Endosperm nearly all absorbed	All soluble in water	None.

Time germinating.	Fatty acid found in same.	Acid soluble in H_2O found in embryo.	Sugar.
hours 24	Traces only	25 c.c. extract neutralised by ·9 c.c. of ·2 per cent. NaHO	Trace.
48	20—25 per cent.	25 c.c. required 1·65 c.c. acid	More.
72	50 per cent., about.	25 c.c. required 2·3 c.c. acid	Fair reaction with Fehling's fluid.
96	70—80 per cent.	25 c.c. required 2·6 c.c. acid	Good reaction.
120	Half residue	Very acid reaction, did not test quantitatively	About as last.
168	None	Strongly acid	About as last.

Two or three points of interest appear in this table. The gradual disappearance of the oil is accompanied by a rise in the quantity of fatty acid up to the fourth day. This then diminishes in turn, and at the end of the period, while still some endosperm is left, both have

disappeared. The amount of material extracted by the ether is about the same for the first three days, when it gradually and regularly diminishes, and at the same time gradually changes its character, becoming much more acid, and containing increasing quantities of water. The endosperm altogether is much more watery at that stage, a certain amount coming away with the ether, and having to be separated by decantation before evaporating the latter. At the conclusion of the period, a considerable amount of acid is left in the endosperm, which is soluble in water and in ether. The quantities of this acid and of the sugar increase up to the fourth day, and then remain fairly constant, a slight further increase only being noticed in the acid. This is to be accounted for by the rapid development of the plant at about that time, the material leaving the endosperm being that at whose expense this growth takes place.

No glycerine could be detected in the endosperms throughout.

In the second set of experiments, in which the endosperms were separated from the embryos, no quantitative estimation was made of the sugar, as its formation coincidently with the disappearance of the oil had been noted continually.

Taking the endosperms alone as shown in the table (p. 385), they bear out the hypothesis based upon the examination of the whole plant. The acid which was dialysable increased in the endosperms up to the fourth day, and then gradually diminished, pointing to an absorption taking place at a rather faster rate than its formation. Taking the whole plant, the acid increased slightly after this time, showing that it was not used in the growing-points quite so rapidly as it was absorbed, but still was undergoing metamorphosis there.

The fact that at the close of the period during which the endosperm supplies nutriment to the embryo only sugar and dialysable acid are present in its cells, besides a little proteid matter, seems a fair indication that these are the bodies into which the reserve material of the oil is transformed for absorption.

The reserve materials of the resting endosperm are thus found to be all replaced by derivatives which are capable of absorption by dialysis.

There is still left a very important question to discuss. Starting with oil and proteids in the resting seed, we find crystallisable acid, sugar, and asparagin passing into the young embryo, and we note intermediate bodies in the shape of the various fatty acids present in castor oil, and of peptone. We find, further, that glycerine which can be liberated from the oil by the ferment, and by laboratory methods, escapes notice however closely it is looked for. What are the probable decompositions that take place, and how can these explain the various products found?

We have clearly first the splitting of the oil into the fatty acids

Table II.—Endosperms only Examined.

Time of germination.	Water present in the endosperm.	Residue from ether extraction.	Oil found in same.	Fatty acid.	Acid soluble in H_2O. Amounts of 0·2 per cent. NaHO required to neutralise 20 c.c. in each case.
hours 24	None	Copious, greasy	Nearly whole bulk	A drop or two on surface of solution of the soap after adding HCl	1 c.c.
48	None	About same amount and character	A little less	Thin scum, not covering the solution of the soap after adding HCl	1·8 c.c.
72	None	As No. 3 ...	Not much ..	Scum covering the solution treated as before	1·4 c.c.
96	A trace ..	Less in amount and losing greasy character	Few drops..	Thin scum on solution	2·15 c.c.
120	More	Still less watery in character	Trace	Curdy precipitate with HCl, floating to top of solution	1·85 c.c.
168	Good deal	Hardly any oily matter in it. Crystallisable matter present	Mere trace..	Less than in last	1·7 c.c.
192	As No. 6 .	No oily matter apparent	None	Mere trace	Did not titrate.

and glycerine. That there is a connexion between the former and the acid which passes into the cotyledon seems certain when we compare the formation of the latter as the former disappears, and remember that the fatty acids themselves are not capable of dialysis. The transformation is not brought about by the agency of the ferment, nor is anything excreted by the cotyledon which will bring about the change. It must not be forgotten that the endosperm is the seat of many processes of oxidation, for, as already stated, Detmer has shown the germination is accompanied by a constant evolution of CO_2.

De Saussure has traced out the same process, showing, too, that there is during the germination a constant absorption of oxygen. Even more light is thrown upon the point by the statement of Godlewski,* that this absorption of oxygen is not, as De Saussure believed, greater than the output of CO_2 throughout the whole period. He states that it is not till the radicle protrudes that the oxygen is taken up in greater quantity, and he points out that as the fat disappears from the seed, the inequality between the oxygen absorbed and the CO_2 exhaled gets less and less. It is just at this period that the fatty acid is being replaced by the dialysable one, as shown in the tables given above. That gentle oxidation of the fatty acids is possible is shown by Hazura and Grüssner,† who state that when alkaline solutions of the liquid fatty acids of castor oil are oxidised by permanganate of potash several derivatives are formed, including some of the lower members of the series of fatty acids. Krafft‡ also states that when ricinoleic acid is oxidised with nitric acid, normal heptylic acid is formed, together with azelaic and oxalic acids. Unfortunately, I have not been able to get sufficient quantity of the acid which is formed during germination to enable me to ascertain its identity. The plant itself at that age is very small, and though the reaction of the acid to litmus-paper is very well marked, the quantities occurring in any case are too small for analysis.

The disappearance of the glycerine is, in all probability, to be associated with the appearance of the sugar. The possible sources of the latter are only three, the fatty acids, the proteids, and the glycerine. That sugar results from the former of these is very unlikely. Theoretically there seems to be a possibility of the change, but no laboratory experiments have yet succeeded in bringing it about. Fischer§ has recently shown that certain acids can, by a process of reduction, give rise to sugar, but these are not such acids as those occurring here. The great probability, too, of the fate of the fatty acids being that suggested above weighs heavily against their furnishing the sugar. The proteids, too, can be accounted for in another way, as appears below. There remains, then, only the glycerine.

Fischer has established the fact that the transformation of glycerine into sugar is possible, and the famous experiments of Luchsinger‖ upon glycerine as an antecedent of glycogen in the course of the hepatic metabolism bear upon the same point. The ready appearance of sugar is, by this hypothesis, accounted for, and the fact that it appears side by side with the fatty acid in the endosperm

* Pringsheim, 'Jahrb. Botan.,' vol. 13, 1882.

† 'Monatsh. f. Chemie,' vol. 9, pp. 475—484.

‡ 'Deutsch. Chem. Ges. Ber.,' vol. 21, 1888, pp. 2730—2737.

§ 'Deutsch. Chem. Ges. Ber.,' vol. 22, p. 2204.

‖ Pflüger's 'Archiv,' vol. 8, 1874, and vol. 18, 1878.

is what would be expected. It is unlikely to be derived from the fatty acid, for the disappearance of a small quantity of this is accompanied by the appearance of a small quantity of the lower acid; it is also unlikely to be derived from the latter, from the fact that both appear synchronously, both increase together, and both are left when the endosperm is finishing its work. Further, the glycerine set free by the decomposition of the oil is sufficient in quantity to account for all the sugar formed.*

There is, of course, another possible alternative. Vines suggests† that the glycerine may undergo oxidation at once with acids, and may be therefore represented by the acids found. This, though possible, seems unlikely, as these last are so much more readily traced to the fatty acid part of the disrupted oil molecule, and especially as we are then reduced to the hypothesis that the sugar comes from the lower acids formed, which has been seen to be improbable.

There is still another theory which needs notice. It is put forward by Vines‡ to explain what Sachs has said, as to starch resulting from the transformation of fat:—"The processes which attend the early stages of the germination of an oily seed may be briefly stated thus: protoplasm undergoes decomposition to form starch, and the continued formation of starch depends upon the reconstruction of protoplasm from the nitrogenous residues of previous decomposition, together with some form of non-nitrogenous organic substance; the non-nitrogenous substance in question is fat." The argument appears unnecessary now that experiments already quoted show that Sachs is in error as to the formation of starch in the endosperm cells during normal germination, but the form of the carbohydrate is not material, and the hypothesis may be advanced to explain the appearance of sugar. It is clear, however, that, if a direct conversion of some antecedent into sugar is possible, it is a much more probable thing than the round-about method suggested. The theory seems called for only to explain an otherwise inexplicable phenomenon, and might as fairly be put forward to explain the appearance of sugar as starch disappears, an appearance which is known to be due to a direct transformation of the latter.

* Since this paper was read, Messrs. Brown and Morris have published in the "Journal of the Chemical Society" (June, 1890) an important research on the germination of some of the Gramineæ. Some of their results have a bearing on the point under notice. They find that excised barley embryoes can be nourished on a solution of glycerine, and under such circumstances a considerable amount of growth takes place in them, which is accompanied by the production of starch in their tissues, just as when they are fed with a solution of sugar. The appearance of carbohydrate at the expense of glycerine is established by their experiments.—J. R. G., July 10, 1890.

† *Op. cit.*, p. 229.

‡ *Op. cit.*, p. 206.

The fate of the resting proteids presents no difficulty. Transformed by the proteolytic ferment into peptone and later into asparagin, we can trace them at once into the cotyledons. There is not a very great store of proteid in the seed; and in the young plant, at any rate before the development of chlorophyll, there is a considerable amount of asparagin. A comparison with the processes in the lupin, where the transformations can be worked out without the complication of the metamorphosis of a large amount of oil, indicates the course of events. In so many other plants, too, the fact of the transport of nitrogenous matter in the form of soluble amides towards the seats of growth has been established, that it seems unnecessary to look for any other form in this case.*

III. *The parts played by the Embryo and the Endosperm respectively in the Process of Germination.*

Allusion has already been made to the statement of Sachs,† that the ferments which cause decompositions in the reserve materials are always formed in the young plant or embryo, and are excreted from the latter into the endosperm. He quotes especially in support of this view the fate of the seeds of *Zea Mais*, and those which, like the date, have their non-nitrogenous reserves laid up in the form of cellulose. Some years before,‡ Gris claimed to have established that the endosperm was, during germination, the seat of an independent life, as much as the embryo was, and was by no means a passive contributor to the latter. Some careful experiments were conducted by Van Tieghem, and published by him in 1877,§ to which no allusion is made by Sachs, but which throw an important light upon this question. Part of his work, like that of Gris, was carried out on *Ricinus*, seeds of which plant were deprived, by careful dissection, of their embryos, and were exposed on damp moss for some weeks to a temperature of 25—30° C. After several days of this exposure he found the isolated endosperms were growing considerably, and at the end of a month they had doubled their dimensions. The change was caused by the enlargement of the constituent cells and the development of air-spaces between them. In the interior of the cells he found the aleurone grains to be gradually dissolving, and the oily matter to be diminishing, though slowly. The dissolution extended throughout the mass of the endosperm, and was not especially prominent on the side that had been nearest to the cotyledons. He

* *Cf.* Sachs, 'Vorlesungen über Pflanzen-Physiologie,' Eng. Trans. by Marshall Ward, p. 346.

† *Op. cit.*, p. 344.

‡ Gris, "Recherches anatomiques et physiologiques sur la Germination." 'Ann. des Sci. Nat.,' Ser. 5, *Bot.*, vol. 2, 1864, p. 100.

§ "Sur la digestion d'Albumen." 'Comptes Rendus,' vol. 84, p. 578.

noted, too, that, though starch did not normally appear in the germinating endosperm, under the condition of non-removal of the products of the decompositions, it did appear in the cells in the form of small grains, though not till after several days. Van Tieghem also observed that the progress of the decompositions could be arrested, and the endosperm made to re-assume a quiescent condition, and that then the aleurone grains again became formed, though in less quantity than before.

To a large extent my observations confirm these of Van Tieghem, though I did not continue the experiment for so long a time. Some endosperms prepared by removal of the embryo by the knife were exposed on damp sand to a temperature of 38° C. in an incubator. Others were placed with them after removal of the plumule and radicle only, leaving the large cotyledons lying on the endosperms undisturbed.

After three days, the former ones contained a little unaltered oil, a good deal of fatty acid, a trace of crystallisable acid, and a little sugar. The latter set were much more swollen than the former, and contained a larger amount of crystallisable acid; the other constituents being much the same.

In a further experiment endosperms, prepared similarly, were attached to the under side of the cork of a small wide-mouthed bottle containing a little water, and were thus cut off from the access of free oxygen. They were then placed in the incubator at 38° C. The change in bulk of these was very slight during the time (fourteen days) during which the experiment lasted; and when examined their contents were found to be much less affected. Part of the oil was transformed, about one-fifth being replaced by fatty acid, while no sugar and no acid soluble in water could be extracted from them.

I cannot confirm Van Tieghem's observation as to the occurrence of starch in the cells under these abnormal conditions; but my experiment, made under the same conditions as his, probably was not conducted for a period long enough to secure its formation. Gris* states that when he found starch grains formed in the endosperm cells it was not until their first contents had been completely absorbed.

Histological investigation of the endosperms, both when germinated normally and when the embryo had been removed, indicates that the changes are not set up by the latter. The decomposition of the oil does not in either case take place regularly on the side nearest to the cotyledons, but cells throughout the whole endosperm are affected simultaneously.

The same conclusion is pointed to by the experiments already quoted as to the antecedent condition of the ferment. The ground

* Gris, *op. cit.*

resting endosperm, when extracted with salt solution or with glycerine, yields up to the solvent something which, though possessing no ferment power, yet is capable of having this developed in it by warming with dilute acid. The cotyledons, on the other hand, are found, even when germination is active, to contain no ferment.

Yet it seems improbable that the function of the young embryo in its relation to the endosperm is one of absorption only. Van Tieghem found that the young endosperm, apart from the embryo, increased in bulk with extreme slowness, not doubling its size till a month had elapsed. In normal germination this result is attained in five or six days. This is not due simply to slowing of the process, owing to elaborated materials not being absorbed. In some of my experiments I laid the flat surfaces of the isolated endosperms upon dialysing paper exposed upon moist sand, so that absorption could take place, but even then the rate of development was scarcely accelerated.

On the other hand, the germination was much more rapid when a small piece of the cotyledon was left adhering to the endosperms, though no removal of products could, under the conditions, take place. The first set of experiments alluded to (p. 375), when seeds with all the embryo taken away were germinated side by side with those that had only lost its axis, shows, too, that the mere presence of the cotyledons, apart from their absorbing power, had a considerable influence on the progress of the germination.

It is difficult to suggest an adequate explanation of this action. As already shown, it is not caused by the formation and subsequent excretion of the glyceride ferment. Nor does it appear that the embryo excretes anything that may bring about the later changes which the ferment does not effect. An embryo extracted from a germinating seed, and washed from adherent matter, was found powerless to effect any change in free ricinoleic acid when the two were placed together in an incubator. Nor would a watery extract of several embryos taken from germinating seeds produce any change in an emulsion of ricinoleic acid in the direction of forming the crystalline dialysable acid found to result from the oxidation of the former. It seems probable that its growth or development acts as a stimulus to the protoplasm of the endosperm cells in which it is embedded, whereby these are caused to undergo their metabolic changes more rapidly than they do in the absence of such stimulus. The processes which are most affected seem to be those dependent on a supply of free oxygen, and not those of the ferment action, a point which supports the idea of the former ones being the expression of the vital activity of the protoplasm of the endosperm cells. Such a stimulus is probably of a physiological character, and is not the mere increase of pressure on the endosperm as the embryo grows.

The investigation of the three points discussed in the foregoing portion of this paper has left unsolved another point of considerable interest, to which my attention was drawn somewhat accidentally. In examining the action of the glyceride ferment upon different emulsions, some experiments were carried out with milk, and there was then found to be present in the germinated seeds not only the two ferments already described, but a third, which acts like rennet. A tube containing 5 c.c. of milk and 2 c.c. of a glyceride extract of germinating endosperms clotted in five minutes when exposed to a temperature of 35° C., a control with the extract boiled remaining unclotted for hours. At the same time a certain amount of acidity was developed in the milk by the action of the glyceride ferment, but the action on the casein was not caused by this. A further experiment was made to establish this conclusion. Two tubes were prepared as before, and their contents coloured with litmus. As soon as clotting had taken place in the one with unboiled extract, dilute HCl was added to the control till it was equally acid with the other, but no clotting or precipitation took place. A further similar set of tubes was prepared, their contents being made slightly alkaline, and on exposure in the incubator the one with unboiled extract clotted, while the control did not.

This ferment, like the other, was by similar experiments shown to be present in an antecedent or zymogen condition in the resting endosperms, and to be capable of conversion in the same manner.

This rennet ferment was found to be most easily extracted by glycerine.

The discussion of the meaning of the rennet ferment here, as in so many plants, must be deferred, pending the completion of further experiments now in progress.

Summary.

The work detailed above leads to the following conclusions :—

1. The reserve materials in the endosperm of *Ricinus communis* consist of oil and proteid matters, the latter being a mixture of globulin and albumose.

2. The changes in germination are partly due to ferment action, there being three ferments present in the germinating seed, one a proteolytic one resembling trypsin; the second a glyceride one, splitting the oil into fatty acid and glycerine; the third a rennet ferment.

3. At least two of these, and therefore, presumably, all of them, are in a zymogen condition in the resting seed, and become active in consequence of the metabolic activity stirred up in the cells by the conditions leading to germination, especially moisture and warmth.

4. The changes caused by the ferment action are followed by others due to the metabolism of the cells, these being processes of weak oxidation.

5. The embryo exercises some influence on the latter, setting up as it develops a stimulus probably of a physiological description.

6. The result of these various processes is to bring about the following decompositions:—

The proteids are by the ferment converted into peptone, and later into asparagin.

The oil is split by the glyceride ferment into fatty acid and glycerine; the latter gives rise to sugar, and the former to a form of vegetable acid, which is soluble in water and in ether, is crystalline, and has the power of dialysis.

7. The mode of absorption is in all cases by dialysis.

8. The appearance of starch and of oil in the embryo or the young plant is due to a secondary formation, and not to a transloca-tion of either.

"A Note on an Experimental Investigation into the Pathology of Cancer." By CHARLES A. BALLANCE and SAMUEL G. SHATTOCK. Communicated by Sir JAMES PAGET, Bart., F.R.S. Received April 15—Read May 1, 1890. Revised June 10, 1890.

Our first method of conducting the enquiry was by seeing if any special micro-organism could be artificially cultivated from malignant tumours, such as can be done from tubercle, and the pathological formations of certain other infective diseases.

These experiments were made in most instances with carcinomata of the breast, and in a manner fully detailed in the 'Pathol. Soc. Trans.,' vol. 38. We thus experimented with three lipomata, one myxoma, three sarcomata, and about thirty carcinomata.

The results yielded by this particular method, and the particular cultivating media mentioned, may be described in a single word as negative.

We have been able to keep portions of many carcinomata sterile for an indefinite time after various periods of incubation up to thirty-three days; and in one case, in which fluid human serum was employed, the incubation was continued for 134 days.

The pieces transferred to solid media which remain sterile undergo no change perceptible to the naked eye. We have at the present time (February 20th, 1890), amongst others, a piece of a